BMDMs isolated from Cav1-/- and WT animals. However, in spite of low caveolin level in HEK293 cells, Kir2.1 expressed in HEK293 cells maintain their cholesterol sensitivity. Furthermore, we also show that Kir currents in Bone-Marrow Derived Macrophages (BMDMs) isolated from Cav1-/- knockout mice are also cholesterol sensitive. Thus, these studies indicate that caveolin-1 and/or intact caveolae are not required for cholesterol sensitivity of Kir channels. Moreover, a single point mutation of Kir2.1, L222I that abrogates the sensitivity of the channels to cholesterol also abolishes their sensitivity to Cav-1 suggesting that the two modulators regulate Kir2.1 channels via a common mechanism.

2378-Pos Board B397

Regulation of a Potassium Channel by the Pro-Domain of a Matrix Metalloprotease

Galina Schmunk[1], Hai M. Nguyen[1], Charles A. Galea[2], Brian J. Smith[3], Robert A. Edwards[1], Raymond Norton[2], George K. Chandy[1].
[1]University of California Irvine, Irvine, CA, USA, [2]Monash University, Melbourne, Australia, [3]La Trobe University, Melbourne, Australia.

Matrix metalloproteases (MMPs) are endopeptidases that regulate diverse biological processes. They are synthesized as zymogens and become active after removal of their prodomains. Much is known about the metalloprotease activity of these enzymes, but non-canonical functions are poorly defined, and functions of the prodomains have been largely ignored. Here we describe a novel metalloprotease-independent, channel modulating function for the pro domain of MMP23 (MMP23-PD). Whole-cell patch clamping and confocal microscopy coupled with deletion analysis demonstrate that MMP23-PD selectively suppresses the voltage-gated potassium channel KV1.3, but not the closely related KV1.2 channel, by trapping the channel intracellularly. Studies with KV1.2-Kv1.3 chimeras suggest that MMP23-PD's channel trapping function requires the presence of Kv1.3's pore domain. NMR studies of MMP23-PD reveal a single, kinked trans membrane α-helix, joined by a short linker to a juxtamembrane α-helix, which is associated with the surface of the membrane and protected from exchange with the solvent. The topological similarity of MMP23-PD to KCNE proteins that co assemble with and modulate KV channels suggests a shared mechanism of channel modulation. MMP23 and KV1.3 expression is overlapping and enhanced in colorectal cancers where the interaction of the two proteins could affect cell function.

2379-Pos Board B398

Modulation of Voltage-Gated K^+ Channels by the Sodium Channel β1 Subunit

Hai M. Nguyen.
University of California, Irvine, Irvine, CA, USA.

Voltage-gated sodium (Nav) and potassium (Kv) channels are key components of neuronal electrical excitability. Here we report that Navβ1, an integral subunit of Nav channels, associates with and modulates the biophysical properties of the Kv1 and Kv7 channels, in an isoform-specific manner, but not those of the Kv3 channels. Distinct domains of Navβ1 modulate specific biophysical properties of different Kv channels. Studies with channel chimeras demonstrate that Navβ1-mediated changes in activation kinetics and voltage-dependence of activation require interaction of Navβ1 with the channel's voltage-sensing domain, while changes in inactivation and deactivation require interaction with the channel's pore domain. A molecular model based on docking studies shows Navβ1 lying in the crevice between the voltage-sensing and pore domains of Kv channels, making significant contacts with the S1 and S5 segments, and the external loop making significant contacts with outer loops of at least two monomers in the channel. Cross-modulation of Nav and Kv channels by Navβ1 may promote diversity and flexibility in the overall control of cellular excitability and signaling.

2380-Pos Board B399

Molecular Determinants of Selectivity for Kv1.3 K^+ Channels

Adam Bartok[1], Gyorgy Panyi[1], Lourival Domingos Possani[2], Zoltan Varga[1].
[1]Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary, [2]Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuervavaca, Mexico.

The voltage-gated Kv1.3 potassium channel plays a key role in the activation of T lymphocytes by maintaining a negative membrane potential. By blocking these channels the proliferation of T cells can be inhibited. Anuroctoxin (AnTX), a 35-amino-acid peptide isolated and characterized previously from the venom of the scorpion *Anuroctonus phaiodactylus,* blocks Kv1.3 with high affinity (Kd = 0.7 nM). Although with lower affinity, the toxin blocks another K^+channel, Kv1.2 (Kd = 6 nM). The aim of the current experiments was to improve the selectivity of AnTx for Kv1.3 by point mutations strategically designed based on sequence and affinity analysis of other K^+ channel blocker toxins. Wild-type and mutant AnTX variants were produced by solid-phase synthesis. Whole-cell patch-clamp was used to measure hKv1.3 hKv1.1, hKv1.2 and hIKCa1 currents. The effect of synthetic wild-type anuroctoxin was similar to that of the natural toxin (Kv1.3: Kd = 0.3 nM and Kv1.2: Kd = 5.3 nM). AnTx F32T practically lost its affinity for Kv1.2 but also showed a slight decrease in affinity for Kv1.3 (Kd = 7.5 nM). The affinity of AnTx N17A for Kv1.3 did not change significantly (Kd = 0.9 nM) and a slight improvement in selectivity could be observed (Kv1.2: Kd = 18.9 nM). Combining the two mutations in one toxin we constructed the mutant N17A,F32T where the advantageous effect of both mutations could be detected. The N17A,F32T mutant is highly selective (no effect on Kv1.2 at 100 nM) and a high affinity blocker of Kv1.3 (Kd = 0.6 nM). In summary, with targeted mutations we designed and produced a selective and high affinity blocker of Kv1.3. Our results provide the foundation for the possibility of the production and future therapeutic application of additional, even more selective toxins.

2381-Pos Board B400

Kv3.4 Channel Expression, Modulation and Function in a Spinal Cord Injury Model of Neuropathic Pain

David Ritter, Benjamin Zemel, Tamara Hala, Angelo Lepore, Manuel Covarrubias.
Thomas Jefferson University, Philadelphia, PA, USA.

We previously showed that Kv3.4 channels underlie most of the high-voltage activated K^+ current (I_{KHV}) in dorsal root ganglion (DRG) nociceptors and elimination of Kv3.4 N-type inactivation by protein kinase C-dependent phosphorylation enhances the channel's ability to repolarize action potentials (APs) (Ritter et al. 2012 *J Physiol* 590:145-61). This function is important as reduction of Kv3.4 channel expression is found in diverse models of neuropathic pain. We hypothesize that phosphorylated Kv3.4 channels underlie an anti-nociceptive homeostatic mechanism in nociceptors that reduces downstream Ca^{++} signaling and down-regulation of Kv3.4 channel expression destroys this putative homeostatic mechanism, contributing to chronic pain. To test this hypothesis, we investigated a spinal cord injury (SCI) rat model of neuropathic pain in which a C5 hemi-contusion induces bilateral forepaw thermal hyperalgesia and mechanical allodynia 2 weeks post injury. In the contralateral SCI DRG, the peak I_{KHV} is reduced by 55% and the time constant of inactivation (Tau_i) increases by 2-fold relative to laminectomy controls. Furthermore, while 85% of laminectomy nocicpetors show a fast inactivating Kv3.4 current, the SCI phenotypes fall into three categories: no current (25%), substantial sustained current (46%) or slow inactivation (29%, Tau_i >2 S.D. of controls). This suggests that Kv3.4 channels are phosphorylated and down-regulated in SCI. Confirming the impact of these changes on AP firing, AP-clamp experiments in SCI nociceptors show altered currents compared to controls. Interestingly, Kv3.4 mRNA splicing is altered in SCI DRG, which might alter the Kv3.4 channels' response to injury. We propose that Kv3.4 channels, their modulation by PKC and regulation of splicing are therapeutic targets in neuropathic pain.
Supported by the Farber Family Foundation (MC), Sigma Xi GIAR (DR), Craig Nielson Foundation (AL), and Paralyzed Veterans of America (AL).

2382-Pos Board B401

Both N- and C-Terminal Interactions Determine the Obligatory KV2.1/KV6.4 Heterotetramerization

Elke Bocksteins, Evy Mayeur, Glenn Regnier, Abbi Van Tilborg, Dirk J. Snyders.
University of Antwerp, Antwerp, Belgium.

Fully assembled voltage-gated potassium (Kv) channel are tetramers of α-subunits. The silent (KvS) channel subunits (Kv5-9) do not form functional homotetramers due to retention in the endoplasmic reticulum (ER). This ER retention is relieved by assembly with Kv2 subunits generating functional Kv2/KvS heterotetramers. For the Kv1-4 subfamilies, the N-terminal T1 domain determines the subfamily specific tetramerization by preventing interactions between subunits that belong to different subfamilies. However, yeast-two-hybrid experiments showed an interaction between the N-termini of Kv6.4 and both Kv2.1 and Kv3.1. We demonstrate here that the subfamily specific Kv2.1/Kv6.4 heterotetramerization is determined by additional C-terminal interactions. Förster Resonance Energy Transfer (FRET) and co-immunoprecipitation (co-IP) using N- and C-terminal Kv6.4 and Kv3.1 fragments as well as N- and C-terminal truncated Kv6.4 and Kv3.1 subunits indicated that both the Kv6.4 N-terminus and S1-S6 segments interact with the corresponding Kv3.1 segments. However, these interactions did not lead to functional Kv3.1/Kv6.4 heterotetramers since co-expression of Kv6.4 with Kv3.1 did not affect the Kv6.4 localization nor the biophysical Kv3.1 properties, and no interaction between Kv3.1 and Kv6.4